PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year)
25 March 2002 (25.03.02)

Applicant's or agent's file reference
BO 42861 AS

IMPORTANT NOTIFICATION

International application No.
PCT/NL00/00697

International filing date (day/month/year)
29 September 2000 (29.09.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
VAN DER MEULEN, Jan
De Sikkel 6
NL-8252 GS Dronten
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
VAN DER MEULEN, Jan
De Sikkel 6
NL-8253 CS Dronten
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
Sylvaine DESCLOUX
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 June 2001 (18.06.01)	
International application No. PCT/NL00/00697	**Applicant's or agent's file reference** BO 42861 AS
International filing date (day/month/year) 29 September 2000 (29.09.00)	**Priority date** (day/month/year) 29 September 1999 (29.09.99)
Applicant KILIAAN, Amanda, Johanne et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   25 April 2001 (25.04.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer S. Mafla (Fax 338.87.40)
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
21 February 2002 (21.02.02)

Applicant's or agent's file reference
BO 42861 AS

IMPORTANT NOTIFICATION

International application No.
PCT/NL00/00697

International filing date (day/month/year)
29 September 2000 (29.09.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
VAN DER MEULEN, Jan
De Lipizzaner 33
NL-8252 GS Dronten
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
VAN DER MEULEN, Jan
De Sikkel 6
NL-8252 GS Dronten
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
Ki-Nam HA
Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/NL 00/00697

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/054 A23L1/0528 A23L1/0526 A61K31/715 A61P3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, FSTA, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 153 013 A (FISONS PLC) 28 August 1985 (1985-08-28) page 2, line 22 -page 4, line 14 page 6, line 1 - line 13 page 7, line 1 - line 22	1-8
X	US 5 260 279 A (GREENBERG NORMAN A) 9 November 1993 (1993-11-09) column 2, line 20 - line 66	1-7
X	EP 0 385 598 A (MORINAGA MILK INDUSTRY CO LTD) 5 September 1990 (1990-09-05) page 2 -page 3, line 20 table 2	1-7
E	WO 00 57717 A (SAMOGGIA SIMONE ;LESEPIDADO S R L (IT)) 5 October 2000 (2000-10-05) the whole document	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

°A° document defining the general state of the art which is not considered to be of particular relevance

°E° earlier document but published on or after the international filing date

°L° document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

°O° document referring to an oral disclosure, use, exhibition or other means

°P° document published prior to the international filing date but later than the priority date claimed

°T° later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

°X° document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

°Y° document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

°&° document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2001	01/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vuillamy, V

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERN ONAL SEARCH REPORT

.nformation on patent family members

In ional Application No
PCT/NL 00/00697

Patent docum nt cited in search report		Publication date	Patent family member(s)		Publication date
EP 0153013	A	28-08-1985	AT	56148 T	15-09-1990
			AU	571641 B	21-04-1988
			AU	3819385 A	08-08-1985
			DE	3579471 D	11-10-1990
			DK	39685 A	02-08-1985
			JP	60188403 A	25-09-1985
US 5260279	A	09-11-1993	AT	120930 T	15-04-1995
			AU	651626 B	28-07-1994
			AU	8603491 A	30-04-1992
			CA	2053933 A	25-04-1992
			DE	69108846 D	18-05-1995
			DE	69108846 T	12-10-1995
			DK	483070 T	17-07-1995
			EP	0483070 A	29-04-1992
			ES	2073154 T	01-08-1995
			JP	4282316 A	07-10-1992
EP 0385598	A	05-09-1990	JP	2222659 A	05-09-1990
			JP	2639726 B	13-08-1997
			DE	69002482 D	09-09-1993
			DE	69002482 T	10-02-1994
			US	4971814 A	20-11-1990
WO 0057717	A	05-10-2000	NONE		

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year)
21 February 2002 (21.02.02)

Applicant's or agent's file reference
BO 42861 AS

IMPORTANT NOTIFICATION

International application No.
PCT/NL00/00697

International filing date (day/month/year)
29 September 2000 (29.09.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
TIMMERMANS, Johannes, Wilhelmus
Schoonenburg 188
NL-6714 GE Ede
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
TIMMERMANS, Johannes, Wilhelmus
Schoonenburg 188
NL-6714 GG Ede
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Col mbettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
Ki-Nam HA
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BO 42861 Dek		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL00/00697	29/09/2000	29/09/1999
International Patent Classification (IPC) or national classification and IPC A23L1/054		
Applicant N.V. NUTRICIA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/04/2001	18.01.2002
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Vuillamy, V Telephone No. +31 70 340 3504

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00697

I. Basis of th report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-9	as originally filed

Claims, No.:

1-8	as originally filed

Drawings, sheets:

1/3-3/3	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-8
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-8
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	

2. Citations and explanations
see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: EP-A-153013
D2: US-A-5260279
D3: EP-A-385598

1. The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-8 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

1.1. The subject-matter of claims 1-8 is not new over D1, which discloses (cf. p.2, l.15 to p.5, l.19; p.7, l.1-22) the use of hydrolyzed dextran having a molecular weight of 10-50000 kDa in nutritional compositions. The viscosity increase mentioned in claim 1 mainly depends on the molecular weight of the dextran and its concentration: D1 discloses dextran of similar molecular weight at concentrations of 0.2-1 g/l and mentions low-viscosity compositions for nasogastric feeding. D1 further mentions reduced absorption of allergic substances in the gut.

1.2. The subject-matter of claims 1-7 is not new over D2, which discloses (cf. col.2, l.20-66) the use of hydrolyzed guar gum having a molecular weight of 20-30 kDa in nutritional compositions. The viscosity increase mentioned in claim 1 mainly depends on the molecular weight of the guar gum and its concentration: D2 discloses hydrolyzed guar gum of similar molecular weight at concentrations of 0.25% and mentions low-viscosity compositions for tube-feeding. D2 further mentions reduced uptake of bacteria or toxins through the mucosal barrier.

1.3. The subject-matter of claims 1-7 is not new over D3, which discloses (cf. p.2, l.1 to p.3, l.20) the use of hydrolyzed konnyaku having a molecular weight of 2-15 kDa in nutritional compositions. The viscosity increase mentioned in claim 1 mainly depends on the molecular weight of the gum and its concentration: D3 discloses hydrolyzed konnyaku of similar molecular weight at concentrations of 1.5 g/l. D3 further mentions reduced absorption of cholesterol.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
BO 42861 AS		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 00/ 00697	29/09/2000	29/09/1999
Applicant		
N.V. NUTRICIA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00697

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/054 A23L1/0528 A23L1/0526 A61K31/715 A61P3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, FSTA, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 153 013 A (FISONS PLC) 28 August 1985 (1985-08-28) page 2, line 22 -page 4, line 14 page 6, line 1 - line 13 page 7, line 1 - line 22	1-8
X	US 5 260 279 A (GREENBERG NORMAN A) 9 November 1993 (1993-11-09) column 2, line 20 - line 66	1-7
X	EP 0 385 598 A (MORINAGA MILK INDUSTRY CO LTD) 5 September 1990 (1990-09-05) page 2 -page 3, line 20 table 2	1-7
E	WO 00 57717 A (SAMOGGIA SIMONE ;LESEPIDADO S R L (IT)) 5 October 2000 (2000-10-05) the whole document	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2001	01/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Vuillamy, V

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 00/00697

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0153013 A	28-08-1985	AT 56148 T	15-09-1990
		AU 571641 B	21-04-1988
		AU 3819385 A	08-08-1985
		DE 3579471 D	11-10-1990
		DK 39685 A	02-08-1985
		JP 60188403 A	25-09-1985
US 5260279 A	09-11-1993	AT 120930 T	15-04-1995
		AU 651626 B	28-07-1994
		AU 8603491 A	30-04-1992
		CA 2053933 A	25-04-1992
		DE 69108846 D	18-05-1995
		DE 69108846 T	12-10-1995
		DK 483070 T	17-07-1995
		EP 0483070 A	29-04-1992
		ES 2073154 T	01-08-1995
		JP 4282316 A	07-10-1992
EP 0385598 A	05-09-1990	JP 2222659 A	05-09-1990
		JP 2639726 B	13-08-1997
		DE 69002482 D	09-09-1993
		DE 69002482 T	10-02-1994
		US 4971814 A	20-11-1990
WO 0057717 A	05-10-2000	NONE	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/33975 A1

(51) International Patent Classification[7]: A23L 1/054, 1/0528, 1/0526, A61K 31/715, A61P 3/02

(21) International Application Number: PCT/NL00/00697

(22) International Filing Date:
29 September 2000 (29.09.2000)

(25) Filing Language: Dutch

(26) Publication Language: English

(30) Priority Data:
1013175 29 September 1999 (29.09.1999) NL

(71) Applicant *(for all designated States except US)*: **N.V. NUTRICIA** [NL/NL]; P.O. Box 1, NL-2700 MA Zoetermeer (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KILIAAN, Amanda, Johanne** [NL/NL]; Harnjesweg 89, NL-6706 AS Wageningen (NL). **GROOT, Jacques, Alphons** [NL/NL]; Heerenweg 153, NL-1851 KN Heiloo (NL). **TIMMERMANS, Johannes, Wilhelmus** [NL/NL]; Schoonenburg 188, NL-6714 GE Ede (NL). **VAN DER MEULEN, Jan** [NL/NL]; De Lipizzaner 33, NL-8252 GS Dronten (NL). **VAN LAERE, Katrien, Maria, Jozefa** [BE/NL]; Kamperfoeliestraat 11, NL-6666 WS Heteren (NL). **BIJLSMA, Pieter, Brandt** [NL/NL]; Elisabeth Wolffstraat 65 e, NL-1043 TS Amsterdam (NL).

(74) Agent: **JORRITSMA, Ruurd**; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

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WO 01/33975 A1

(54) Title: NUTRITIONAL COMPOSITIONS WHICH CONTAIN NON-DIGESTIBLE POLYSACCHARIDES AND USE THEREOF TO REDUCE TRANSPORT THROUGH TIGHT JUNCTIONS

(57) Abstract: The present invention relates to the use of one or more non-digestible polysaccharides selected from the group consisting of dextrans having a molecular weight of 8 kD to 40,000 kD, hydrolysed (gluco)mannans having a molecular weight of 0.5 kD to 1,000 kD and hydrolysed (galacto)mannans having a molecular weigth of 0.5 kD to 1,000 kD for the preparation of a nutritional composition to reduce the uptake of high molecular weight substances, allergens and microorganisms through the intestinal wall, more particularly to reduce transport of high molecular weight substances, allergens and microorganisms through the tight junctions in the intestines, the rise in the viscosity of the nutritional composition caused by the polysaccharides being less than 20 mPa.s. The nutritional compositions can be used to prevent or to treat allergy, allergic reactions, sepsis and inflammatory processes, such as can arise under emotional and physical stress, ischaemia, reperfusion damage during and after operations, after radiation treatment and/or chemotherapy of cancer patients and in the case of inflammatory diseases of the intestine, diarrhoea and allergies.

Nutritional compositions which contain n n-digestible p lysaccharides and use thereof to reduce transport thr ugh tight juncti ns

The present invention relates to nutritional compositions which contain a specific class of non-digestible dextrans, hydrolysed (galacto)mannans and/or hydrolysed (gluco)mannans. These compositions reduce the uptake of high molecular weight substances, allergens and microorganisms through the intestinal wall. In particular, the present invention relates to reduction of the free transport of such substances through the tight junctions (TJ) of the intestines, without the transport of low molecular weight substances, such as nutrients, via the intestinal epithelium being impeded. The compositions can be used to prevent the increased permeability of the intestinal wall, resulting from various causes, and the penetration of toxins, antigens and pathogenic microorganisms present in the lumen which is caused as a result.

The structure and function of tight junctions is described, inter alia, in Ann. Rev. Physiol. 60, 121-160 (1998) and in Ballard T.S. et al., Annu.Rev.Nutr., 1995, 15:35-55. Tight junctions do not form a rigid barrier but play an important role in the diffusion through the intestinal epithelium from lumen to bloodstream and vice versa.

The permeability of the tight junctions is highly regulated and can be disturbed by illness and certain toxins in the lumen. Regulation takes place from the nervous system, the hormonal system and the immune system. When the tight junctions open, substances which have a high molecular weight, allergens and even microorganisms will pass through the tight junctions. The translocation of substances having a high molecular weight can under certain circumstances give rise to sensitisation of the immune system and result in allergic reactions on subsequent exposure. Translocation of pathogenic microorganisms imposes greater strain on the immune system and can make persons and animals ill, inter alia in periods of lowered resistance. The same applies, for example, in the case of bacterial toxins which have been able to pass through the epithelial layer and have been able to reach the bloodstream.

The invention now relates to the use of one or more non-digestible polysaccharides selected from the group consisting of dextrans having a molecular weight of 8 kD to 40,000 kD, hydrolysed (gluco)mannans having a molecular weight of 0.5 kD to 1,000 kD and hydrolysed (galacto)mannans having a molecular weight of 0.5 kD to 1,000 kD to reduce the uptake of high molecular weight substances, allergens and microorganisms

through the intestinal wall, with the proviso that the rise in the viscosity of the nutritional composition caused by the polysaccharides is less than 20 mPa.s.

More particularly, the invention relates to the use of the abovementioned compositions to reduce transport of high molecular weight substances, allergens and microorganisms through the tight junctions in the intestines.

In addition to reducing the transport of harmful substances and microorganisms to a significant extent, a significant advantage of the present invention is that the normal transport of useful substances (nutrients) such as glucose, amino acids, dipeptides or trace elements is virtually maintained.

According to the invention non-digestible polysaccharides are understood to be polysaccharides which are not, or are barely, digested or converted by the human digestive enzymes under the conditions prevailing in the body. It should be pointed out that some of the non-digestible polysaccharides can be fermented by the microorganisms present in the intestines (colon, caecum and part of the ileum). Without wishing to be tied to any theory, it is, however, expected that the effect of the polysaccharides on the paracellular transport does not take place via the fermentation products.

The degree to which the polysaccharides are digested can be established using the method as described in Minekus, M., Ph.D. Thesis, University of Utrecht, 1998, Development and validation of a dynamic model of the gastrointestinal tract, Section 2. The polysaccharides according to the invention are less than 50% digestible and preferably less than 30% digestible.

Dextrans according to the invention are understood to be dextrans obtained via a (bio)synthetic route or naturally occurring dextrans. The molecular weight of such dextrans can be regulated by partial acid or enzymatic hydrolysis of the molecule followed by repeated fractionation and precipitation with alcohol or ultrafiltration. These methods, which are known per se to those skilled in the art, must be carried out in such a way that the molecular weight of the dextrans falls within the cited range of 8 kD to 40,000 kD.

Dextrans having a molecular weight of 20 kD to 2,000 kD are preferably used.

The term (gluco)mannans is used to refer both to the mannans and the glucomannans. The same applies in the case of the (galacto)mannans. Examples of galactomannans are guar gum, locust bean gum and tara gum. These (galacto)mannans and (gluco)mannans are used in the hydrolysed form. The molecular weights are between 0.5 kD and 1,000 kD.

Mixtures of dextrans, (galacto)mannans and (gluco)mannans can also be used.

The hydrolysed (galacto)mannans or (gluco)mannans according to the invention can be obtained by partial, but extensive, hydrolysis, for example with the aid of enzymes suitable for this purpose, by means of which substantial quantities of oligosaccharides having a chain length of 3 to 5,600, preferably of 4 to 1,000, are produced.

The polysaccharides are preferably present in the preparation in an amount such that the concentration of these polysaccharides in the intestines is 0.1 to 20 g/l, preferably 0.5 to 10 g/l and preferentially 1 to 6 g/l. The minimum quantity of the active ingredient is determined in that a significant decrease in the transport through the tight junctions is detected.

It is not necessary for the polysaccharides to be administered at that location where the paracellular transport is disturbed. The presence of the active component at a location somewhere in the intestines between the stomach and the affected location is sufficient.

Some of the polysaccharides used according to the invention have a viscosity-increasing action which could prevent the absorption of nutritional components. The preparation must have a composition such that the normal transcellular transport is not impeded.

More particularly, the nutritional composition according to the invention has a viscosity of less than 100 mPa.s, preferably less than 40, but even more preferentially less than 30 mPa.s. For the present invention it is important in particular that the polysaccharides, independently of the other constituents of the composition, have only a low viscosity-increasing effect. The viscosity-increasing effect of the active polysaccharides in the composition must be less than 20 and preferably less than 10 mPa.s and can be, for example, 3 mPa.s. Thus, the major proportion of the viscosity of the product is caused by components other than the polysaccharides in the product.

The viscosity is determined by means of a Carri-med at a shear rate of 100 per second and at 20°C.

In the case of dry products the viscosity limits described above apply after reconstitution of the product.

In general, therefore, the type of polysaccharide (molecular weight) and the concentration thereof will be so chosen that an optimum combination of effectiveness and viscosity is obtained. Not only molecule size, but also degree of branching and degree of loading determine action, viscosity and/or fermentation behaviour.

The polysaccharides according to the invention prevent the free transport of high molecular weight substances, allergens and microorganisms through the tight junctions of the intestinal wall. In this context high molecular weight substances are understood to be the substances which under normal conditions are not able to pass through the tight junctions, or are able to do so only in minor amounts, and which can be assumed to have a toxic or allergenic action. These substances will in general have a molecular size of above 4,000 Dalton. Antigens, substances which activate the immune system, are in general peptides, which may or may not have been glycosided, frequently with a molecular weight of more than 10,000 Dalton. Allergens are antigens which produce an allergic reaction which usually is mediated via immunoglobulin E.

In this context microorganisms are understood to be in particular microorganisms which occur in the intestinal lumen. Thus, for example, under certain conditions overgrowth of microorganisms can take place in the small intestine, as a result of which tight junctions are to an increased extent exposed to these microorganisms.

According to another aspect of the invention foods or preparations are proposed which contain these non-digestible polysaccharides. These foods can be:

- complete foods;
- food supplements;
- health-promoting preparations; and
- tube feeds.

The compositions according to the invention can be used to prevent or to treat specific types of allergy, allergic reactions, sepsis and inflammatory processes, such as can arise under emotional and physical stress, ischaemia, reperfusion damage during and after operations, after radiation treatment and/or chemotherapy of cancer patients and in the case of inflammatory diseases of the intestine, diarrhoea and allergies.

The complete foods and food supplements described above can in particular be used in the treatment of, or to prevent, inflammatory diseases of the intestines, such as colitis ulcerosa, inflammatory bowel disease and Crohn's disease. Specific other constituents which can be incorporated in such foods and supplements are growth hormones, glutamine, n-3 LCPUFAs and the requisite contents of macro- and microingredients.

Furthermore, the foods according to the invention can be used before and after operations. Specifically, ischaemia and reperfusion damage to the intestine often occur during operations, as a result of which the tight junctions open. Introducing the

polysaccharides according to the invention into the intestines before and after the operation could prevent the uncontrolled paracellular transport. The administration of these polysaccharides can also be beneficial after chemotherapy.

In the case of diarrhoea a number of pathomorphological changes can also arise which are associated with an increased permeability of the tight junctions. These changes can arise with specific types of diarrhoea. The complete foods and food supplements according to the invention can be used to counteract the adverse consequences of this increased permeability.

The tight junctions can also open during stress, both of a physical nature (for example endurance sports) and of an emotional nature, as a result of which bacterial translocation takes place. Examples of emotional stress under which this takes place is the stress which arises during the transport of pigs to the slaughter house. Contamination of the meat can occur as a result. Another example is the stress that occurs when weaning piglets. The polysaccharides can be administered before the stress takes place, during stress or after the stress has taken place.

With the aid of the polysaccharides according to the invention it is also possible to prepare preparations which are suitable for patients who have a food allergy, such as an allergy to cow's milk or to gluten. The increase in the permeability as a result of exposure to the allergen can be prevented. These preparations are of such composition that they do not contain the said allergens.

The invention is explained on the basis of the following examples and with reference to the appended figures, in which

Figure 1 shows the Ussing chamber used in the examples;

Figure 2 shows the inhibition of the effects of caprate by dextran;

Figure 3 shows the inhibition by hydrolysed tara gum of the increased paracellular permeability caused by melitin;

Figure 4 shows the effect of dextrans on the HRP flow;

Figure 5 shows the effect of dextrans on the HRP flow in a pig under anaesthesia;

Figure 6 shows the effect of dextran on the increased permeability of the intestine of a microvillus inclusion patient.

Examples

I Examples of pr ducts

Examples of compositions of various types of products in which the active component is dextran are given below.

The various types of product can be complete enteral foods, for use by the patient him or herself or for use as a tube feed. The product can be either in liquid form or in powder form, which is ready for use after dissolving. The active components can also be used as an ingredient in another food (for example bread) or in food supplements, such as a bar, a dairy product such as yoghurt, or a powder in the form of a sachet.

Example 1

Ready-to-feed, liquid, complete food for use before or after operations.

The composition is as follows per 100 ml of the product:

Protein:	7.0 g
Fat:	4.0 g
Carbohydrate:	21 g
Dextran:	0.2 g

Minerals in a quantity of 1/15th of the recommended daily allowance (= RDA) can be added per 100 ml of the product. Trace elements and vitamins are added in somewhat larger amounts, i.e. 2/15 RDA. The product is of a composition such that 1,500 ml has to be consumed by the patient.

Example 2

Complete tube feed for persons suffering from inflammatory bowel disease. Per 100 ml the product contains:

Protein based on casein 7.0 g

Fat based on vegetable oils and 10% fish oil and 20% MCT; the linoleic acid content is 20% and the alpha-linolenic acid content 4.5%

Premixes containing the conventional forms of trace elements, vitamins and minerals Na, K, Ca, Mg, P, Zn, Fe, Mn, Cu, vit. B1, B2, niacin, A, D, K, B6, B12, pantothenic acid, folic acid.

Dextran: 0.6 g

Example 3

Food supplement for patients suffering from food intolerance or allergy.

Yoghurt based on soya milk. Per 100 ml the yoghurt contains:

Protein 4.0 g, fat 3.9 g, carbohydrates 12.3 g and 0.1 RDA of vitamins and trace elements.

Na = 80; K = 135; Cl = 125; Ca = 50; P = 50; Mg = 20 mg

Hydrolysed galactomannans 0.5 g

Example 4

Energy drink for athletes.

Per 100 ml the liquid contains

Carbohydrate:	7.0 g
Glucose:	0.2 g
Fructose:	1.8 g
Lactose:	0.4 g
Sucrose:	1.7 g
Polysaccharides:	2.5 g
Organic acids:	0.4 g
Minerals:	
Na:	37 mg
K:	17 mg
Cl:	58 mg
Ca:	8 mg
Mg:	1 mg
Vitamin C:	15 mg
Dextran:	0.1 g

Example 5

Premix for use in pig or piglet feed.

A/Premix consisting of 90% cornflour and 10% 150 kD dextran

B/Premix consisting of a suitable premix of vitamins, trace elements and minerals and 10% dextran.

Premix A or B, or mixtures thereof, can be used in the production of pig feeds. These can

be special feeds for use when pigs are transported, have to be rehoused in the sty or if they have a period of lowered resistance.

The premixes can also be used in a piglet feed for use after weaning, as an additive or instead of the premixes which are already known for use in piglet feed.

II Effect on transport via the tight junctions of the intestine

Use was made of a model set-up for determination of the effect of the polysaccharides used.

A test animal, such as a rat or guinea pig, was brought under narcosis. The stomach wall was then opened and a piece of the ileum tied off. The intestinal tissue was removed and stripped of layers of muscle. The preparation thus obtained was then stretched between two compartments through which oxygenated solutions flowed (Figure 1). The preparation was treated either with buffer (control or blank) or caprate in buffer in order to open the tight junctions (100% permeability) or with the combination of caprate and a certain concentration of polysaccharide in buffer. As a measure of the permeability the transport of HRP (horseradish peroxidase) over the preparation was determined in accordance with known methods.

The results of this type of experiments are shown in Figures 2 to 5.

The in vitro effect of dextran (70 kD) on the increased HRP flow caused by caprate in a guinea pig intestinal epithelium is shown in Figure 2.

The in vitro effect of hydrolysed tara gum (900 D) on the HRP flow of Caco-2 cells under the influence of 2 μM melitin is shown in Figure 3. It can be seen that the increased paracellular permeability caused by melitin is inhibited by tara gum.

The effect of various dextrans on the HRP flow of Caco-2 cells under the influence of 2 μM melitin is shown in Figure 4. Phar in the figure stands for Pharmacosmos.

Figure 5 shows the effect of dextran on the increased HRP flow in the pig intestine caused by ischaemia. The figure relates to experiments with a pig under full narcosis, in which segments of the caudal section of the jejunum were taken. The effect of 5.6 g/l dextran (70 kD)(D) in the in situ ischaemia reperfusion model in pigs as a function of the duration of ischaemia was determined in comparison with control (C) where no dextran was introduced into the lumen during ischaemia. A significant fall in the HRP flow under the influence of dextran was found compared with the control value.

Suction biopsies were taken from the duodenum of a child suffering from

microvillus inclusion disease (MVID). In the Ussing chamber these preparations displayed a four-fold increase in permeability to HRP compared with the normal value. After adding 70 kD dextrans to the luminal compartment of the Ussing chamber to give a concentration of 4.2 g/l the permeability was reduced to the normal level. No further HRP could be detected in the paracellular spaces or tight junctions by means of electron microscopy. A corresponding result was obtained with dextrans having a molecular weight of 150 kD.

Figure 6 shows the result of this experiment with dextrans having a molecular weight of 70 kD. After 120 minutes a clear difference is detectable in the permeability with and without the addition of dextrans.

Claims

1. Use of one or more non-digestible polysaccharides selected from the group consisting of dextrans having a molecular weight of 8 kD to 40,000 kD, hydrolysed (gluco)mannans having a molecular weight of 0.5 kD to 1,000 kD and hydrolysed (galacto)mannans having a molecular weight of 0.5 kD to 1,000 kD for the preparation of a nutritional composition to reduce the uptake of high molecular weight substances, allergens and microorganisms through the intestinal wall, with the proviso that the rise in the viscosity of the nutritional composition caused by the polysaccharides is less than 20 mPa.s.

2. Use according to Claim 1, wherein the polysaccharides are selected from dextrans having a molecular weight of 20 kD to 2000 kD.

3. Use according to one of the preceding claims, wherein the polysaccharides are contained in the composition in an amount such that the concentration of these polysaccharides in the intestine is 0.1 to 20 g/l, preferably 0.5 to 10 g/l and preferentially 1 to 6 g/l.

4. Use according to one of the preceding claims, wherein the nutritional composition is in the form of a complete food.

5. Use according to one of Claims 1 to 3, wherein the nutritional composition is in the form of a food supplement.

6. Use according to one of the preceding claims to reduce transport of high molecular weight substances, allergens and microorganisms through the tight junctions in the intestines.

7. Use according to one of the preceding claims, to prevent or to treat allergy, allergic reactions, sepsis and inflammatory processes, such as can arise under emotional and physical stress, ischaemia, reperfusion damage during and after operations, after radiation treatment and/or chemotherapy of cancer patients and in the case of inflammatory diseases

of the intestine, diarrhoea and allergies.

8. Nutritional composition which contains dextrans having a molecular weight of 8 kD to 40,000 kD, with the proviso that the rise in the viscosity of the nutritional composition caused by the dextrans is less than 20 mPa.s.

Fig 1

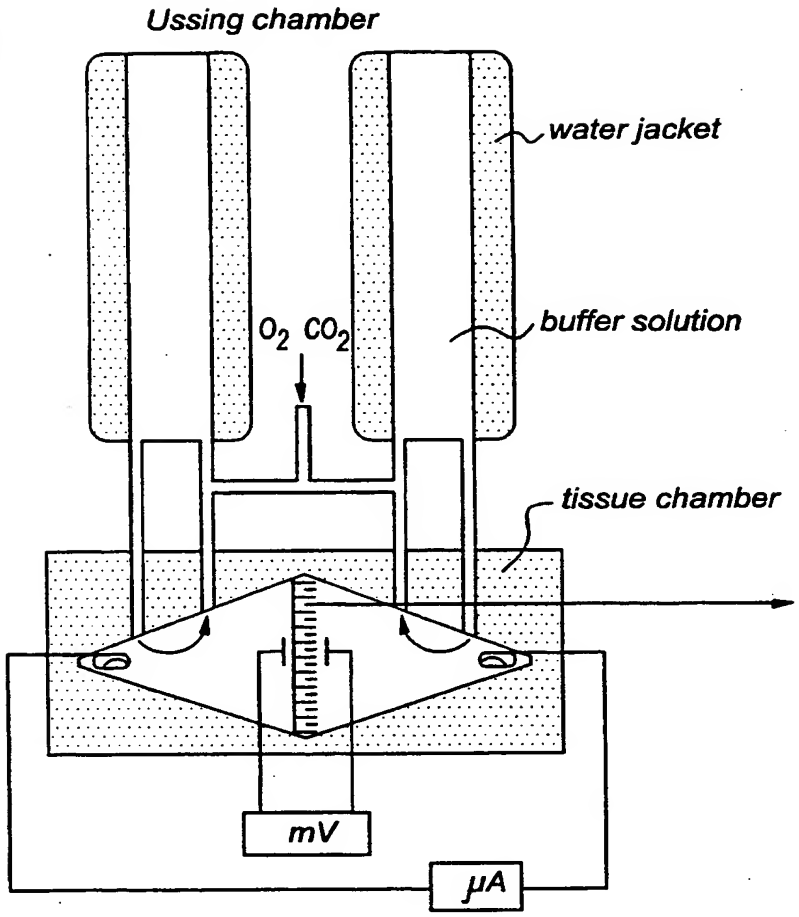

Ussing chamber
water jacket
buffer solution
O_2 CO_2
tissue chamber
mV
µA

Fig 2

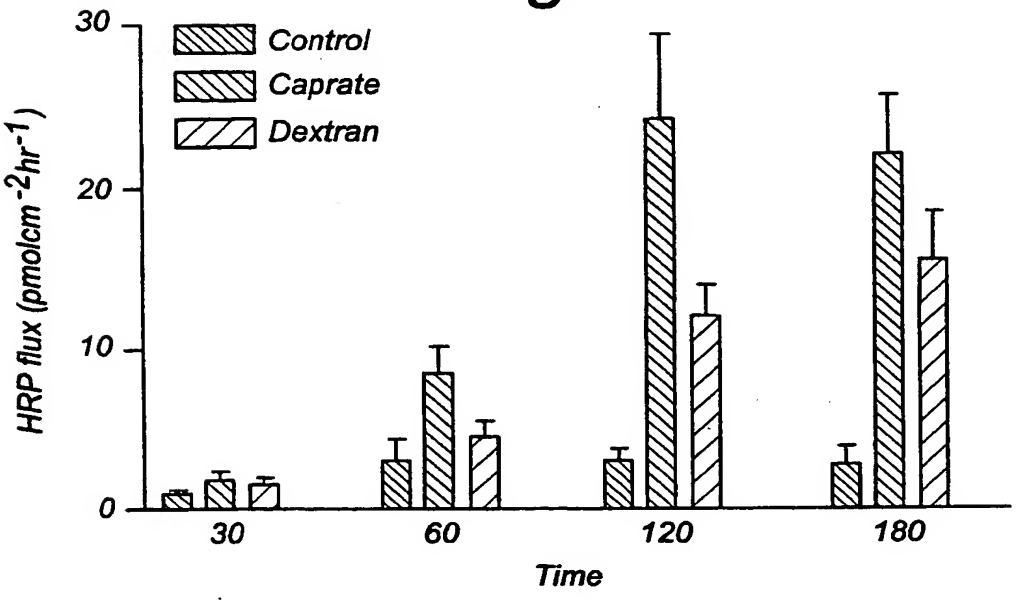

30
20
10
0
Control
Caprate
Dextran
HRP flux (pmolcm^{-2}hr^{-1})
30
60
120
180
Time

Fig 3

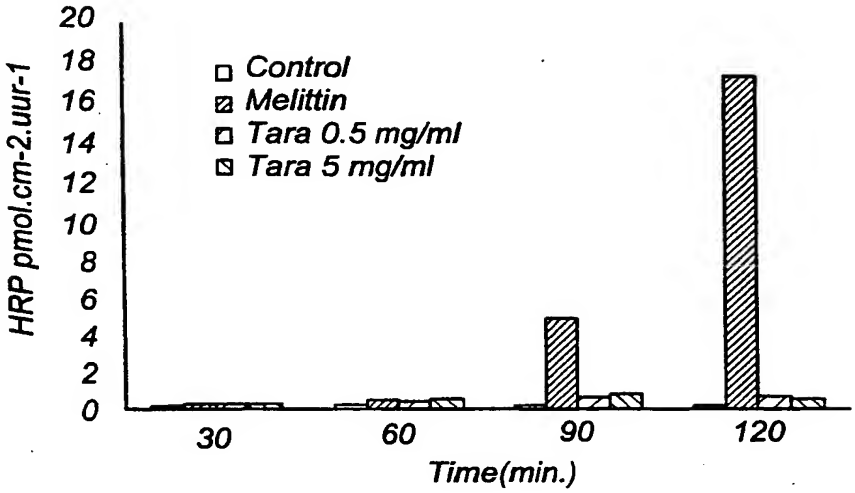
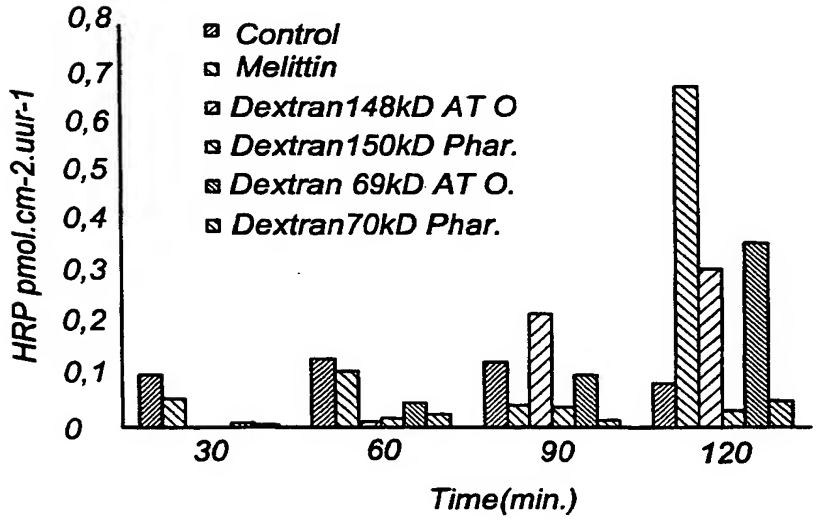

HRP pmol.cm-2.uur-1
20
18
16
14
12
10
8
6
4
2
0
Control
Melittin
Tara 0.5 mg/ml
Tara 5 mg/ml
30
60
90
120
Time(min.)

Fig 4


HRP pmol.cm-2.uur-1
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0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Control
Melittin
Dextran148kD AT O
Dextran150kD Phar.
Dextran 69kD AT O.
Dextran70kD Phar.
30
60
90
120
Time(min.)

Fig 5

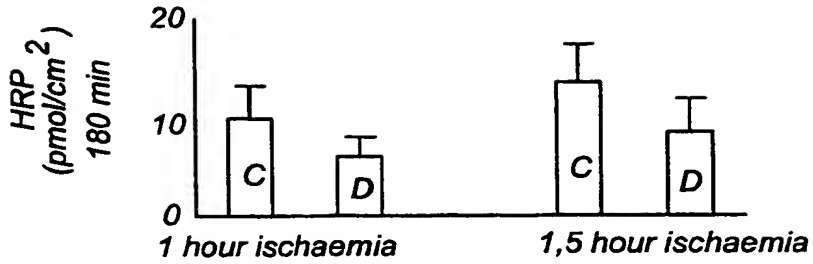
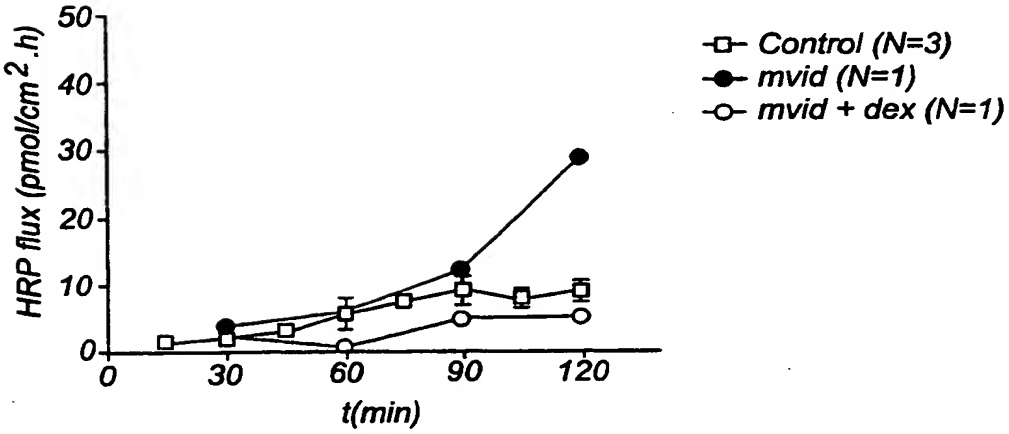

HRP (pmol/cm2) 180 min
20
10
0
C
D
C
D
1 hour ischaemia
1,5 hour ischaemia

Fig 6


HRP flux (pmol/cm2.h)
50
40
30
20
10
0
0
30
60
90
120
t(min)
Control (N=3)
mvid (N=1)
mvid + dex (N=1)

INTERNATIONAL SEARCH REPORT

Int[illegible]nal Application No
PCT/NL 00/00697

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/054 A23L1/0528 A23L1/0526 A61K31/715 A61P3/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, FSTA, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 260 279 A (GREENBERG NORMAN A) 9 November 1993 (1993-11-09) column 2, line 20 - line 66	1-7
X	EP 0 385 598 A (MORINAGA MILK INDUSTRY CO LTD) 5 September 1990 (1990-09-05) page 2 -page 3, line 20 table 2	1-7
E	WO 00 57717 A (SAMOGGIA SIMONE ;LESEPIDADO S R L (IT)) 5 October 2000 (2000-10-05) the whole document	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2001	01/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Vuillamy, V

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 00/00697

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WO 0057717 A	05-10-2000	NONE	

PCT

REQUEST

03. 11. 00

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT/NL 00 / 00697

International Filing Date 29 SEP 2000 (29. 09. 00)

Name of receiving Office and "PCT International Application": BUREAU VOOR DE INDUSTRIËLE EIGENDOM PCT. INTERNATIONAL APPLICATION

Applicant's or agent's file reference (if desired) (12 characters maximum): BO 42861 AS

Box No. I TITLE OF INVENTION Nutritional compositions which contain non-digestible polysaccharides and use thereof to reduce transport through tight junctions

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: The Netherlands (NL)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

JORRITSMA, Ruurd et al
Nederlandsch Octrooibureau
Scheveningseweg 82, P.O. Box 29720
NL-2502 LS THE HAGUE
THE NETHERLANDS

Telephone No. 70 3527500

Facsimile No. 70 3527528

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

C ntinuation f Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GROOT, Jacques Alphons
Heerenweg 153
NL-1851 KN HEILOO
The Netherlands

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: The Netherlands (NL)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

TIMMERMANS, Johannes Wilhelmus
Schoonenburg 188
NL-6714 GE EDE
The Netherlands

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: The Netherlands (NL)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

VAN DER MEULEN, Jan
De Lipizzaner 33
NL-8252 GS DRONTEN
The Netherlands

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: The Netherlands (NL)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

VAN LAERE, Katrien Maria Jozefa
Kamperfoeliestraat 11
NL-6666 WS HETEREN
The Netherlands

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: ~~The Netherlands (NL)~~ Belgium (BE)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BIJLSMA, Pieter Brandt
Elisabeth Wolffstraat 65 e
NL-1043 TS AMSTERDAM
The Netherlands

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: The Netherlans (NL)

State *(that is, country)* of residence: The Netherlands (NL)

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ■ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ■ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ■ **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ■ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ■ **AE** United Arab Emirates
- ■ **AG** Antigua and Barbuda
- ■ **AL** Albania .....
- ■ **AM** Armenia .....
- ■ **AT** Austria .....
- ■ **AU** Australia .....
- ■ **AZ** Azerbaijan
- ■ **BA** Bosnia and Herzegovina .....
- ■ **BB** Barbados
- ■ **BG** Bulgaria .....
- ■ **BR** Brazil .....
- ■ **BY** Belarus .....
- ■ **BZ** Belize
- ■ **CA** Canada
- ■ **CH and LI** Switzerland and Liechtenstein
- ■ **CN** China .....
- ■ **CR** Costa Rica .....
- ■ **CU** Cuba .....
- ■ **CZ** Czech Republic .....
- ■ **DE** Germany .....
- ■ **DK** Denmark .....
- ■ **DM** Dominica
- ■ **DZ** Algeria .....
- ■ **EE** Estonia .....
- ■ **ES** Spain .....
- ■ **FI** Finland .....
- ■ **GB** United Kingdom
- ■ **GD** Grenada
- ■ **GE** Georgia .....
- ■ **GH** Ghana .....
- ■ **GM** Gambia
- ■ **HR** Croatia .....
- ■ **HU** Hungary .....
- ■ **ID** Indonesia
- ■ **IL** Israel .....
- ■ **IN** India .....
- ■ **IS** Iceland
- ■ **JP** Japan .....
- ■ **KE** Kenya .....
- ■ **KG** Kyrgyzstan .....
- ■ **KP** Democratic People's Republic of Korea .....
- ■ **KR** Republic of Korea .....
- ■ **KZ** Kazakhstan .....
- ■ **LC** Saint Lucia
- ■ **LK** Sri Lanka
- ■ **LR** Liberia
- ■ **LS** Lesotho .....
- ■ **LT** Lithuania
- ■ **LU** Luxembourg
- ■ **LV** Latvia
- ■ **MA** Morocco .....
- ■ **MD** Republic of Moldova .....
- ■ **MG** Madagascar .....
- ■ **MK** The former Yugoslav Republic of Macedonia .....
- ■ **MN** Mongolia
- ■ **MW** Malawi .....
- ■ **MX** Mexico .....
- ■ **MZ** Mozambique
- ■ **NO** Norway
- ■ **NZ** New Zealand .....
- ■ **PL** Poland .....
- ■ **PT** Portugal .....
- ■ **RO** Romania
- ■ **RU** Russian Federation .....
- ■ **SD** Sudan
- ■ **SE** Sweden
- ■ **SG** Singapore
- ■ **SI** Slovenia .....
- ■ **SK** Slovakia .....
- ■ **SL** Sierra Leone .....
- ■ **TJ** Tajikistan .....
- ■ **TM** Turkmenistan .....
- ■ **TR** Turkey .....
- ■ **TT** Trinidad and Tobago .....
- ■ **TZ** United Republic of Tanzania
- ■ **UA** Ukraine .....
- ■ **UG** Uganda .....
- ■ **US** United States of America .....
- ■ **UZ** Uzbekistan .....
- ■ **VN** Viet Nam .....
- ■ **YU** Yugoslavia .....
- ■ **ZA** South Africa .....
- ■ **ZW** Zimbabwe .....

Check-box reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

 PCT/NL 00 / 00697

Box N . VI PRIORITY CLAIM ☐ Further priority claim[illegible]e indicated in the Supplemental Box.

Filing date f earlier application (day/month/year)	Number of earlier application	Where earlier application is: nati nal application: country	regional application:* regional Office	international application: receiving Office
item (1) 29.09.99		the		
29 September 1999	1013175	Netherlands		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EPA

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)*	Number	Country *(or regional Office)*
24 May 2000	SN 34155 NL	The Netherlands

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	: 5
description (excluding sequence listing part)	:9
claims	:2
abstract	:1
drawings	: 3
sequence listing part of description	:
Total number of sheets	: 20

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: copy search report

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

GROENEVELD, Yme G.

Nederlandsch Octrooibureau, The Hague, 29 September 2000

For receiving Office use only

1. Date of actual receipt of the purported international application:	29.09.00 29 SEP 2000	2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.	

For International Bureau use only

Date of receipt of the record copy by the International Bureau: 09 NOVEMBER 2000 09 NOV 2000

Fig 1

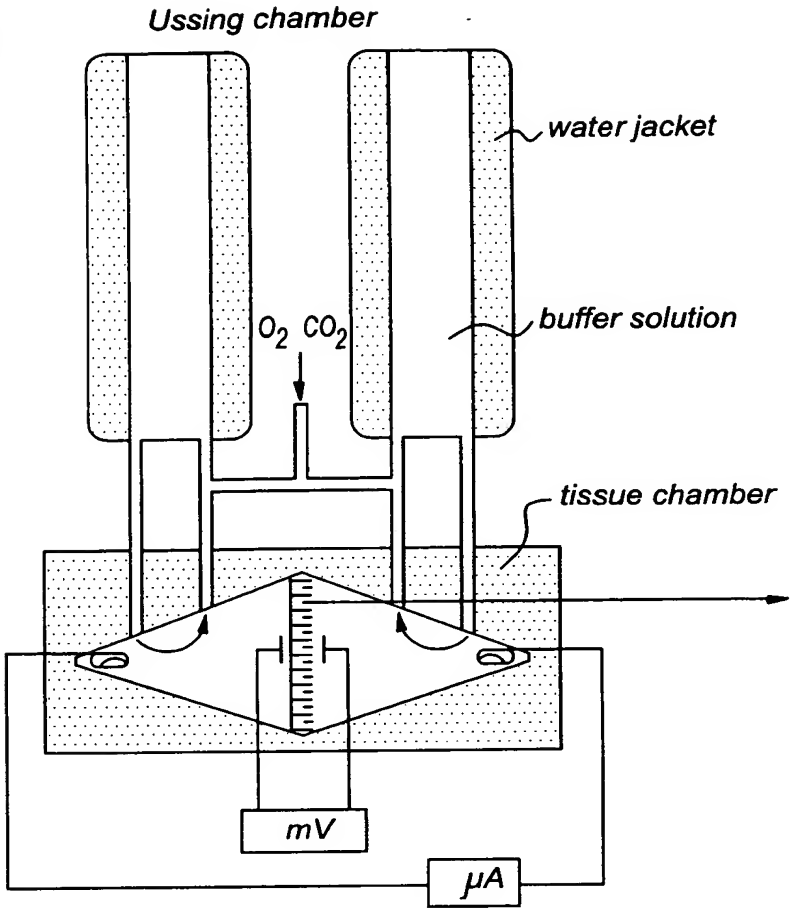

Ussing chamber
water jacket
O_2 CO_2
buffer solution
tissue chamber
mV
µA

Fig 2

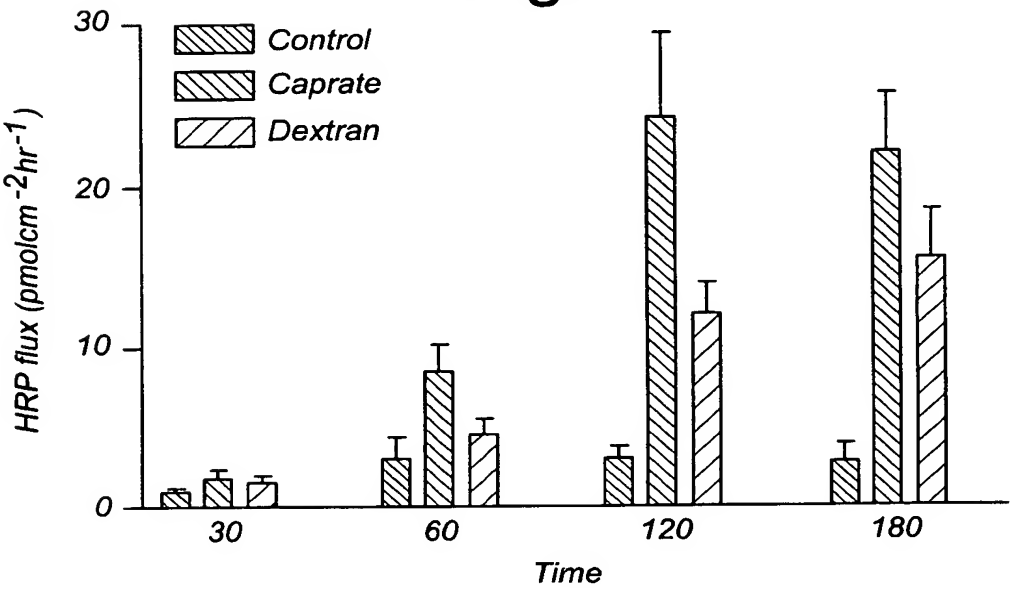

30
20
10
0
Control
Caprate
Dextran
HRP flux (pmolcm^{-2}hr^{-1})
30
60
120
180
Time

Fig 3

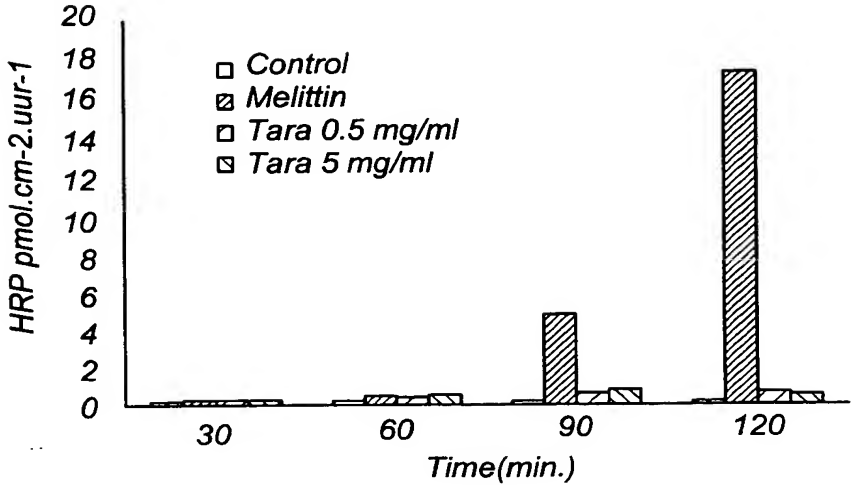

20
18
16
14
12
10
8
6
4
2
0
HRP pmol.cm-2.uur-1
Control
Melittin
Tara 0.5 mg/ml
Tara 5 mg/ml
30
60
90
120
Time(min.)

Fig 4

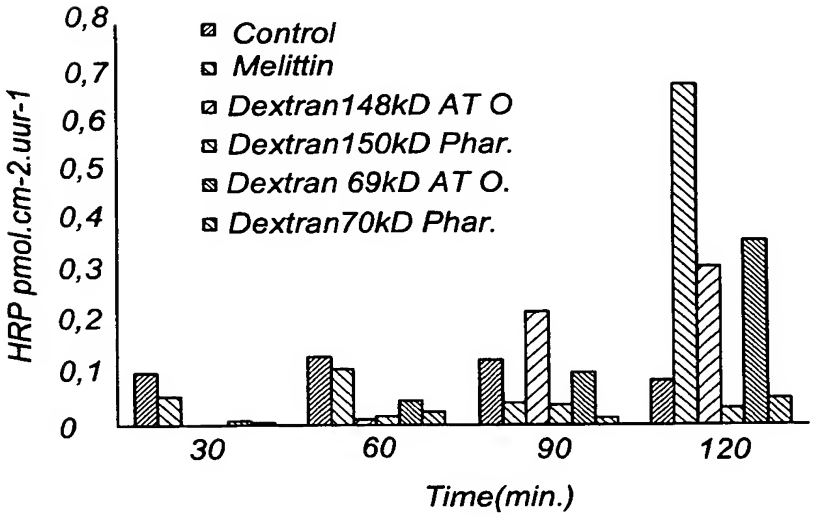

0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
HRP pmol.cm-2.uur-1
Control
Melittin
Dextran148kD AT O
Dextran150kD Phar.
Dextran 69kD AT O.
Dextran70kD Phar.
30
60
90
120
Time(min.)

Fig 5

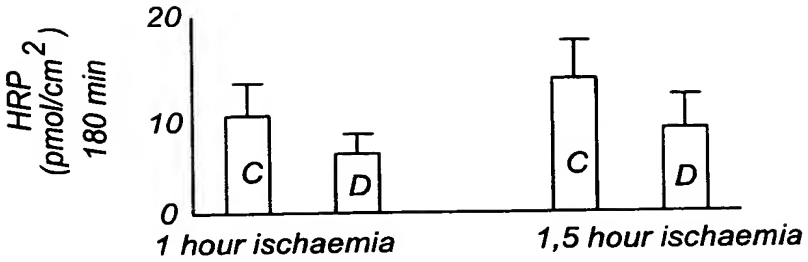
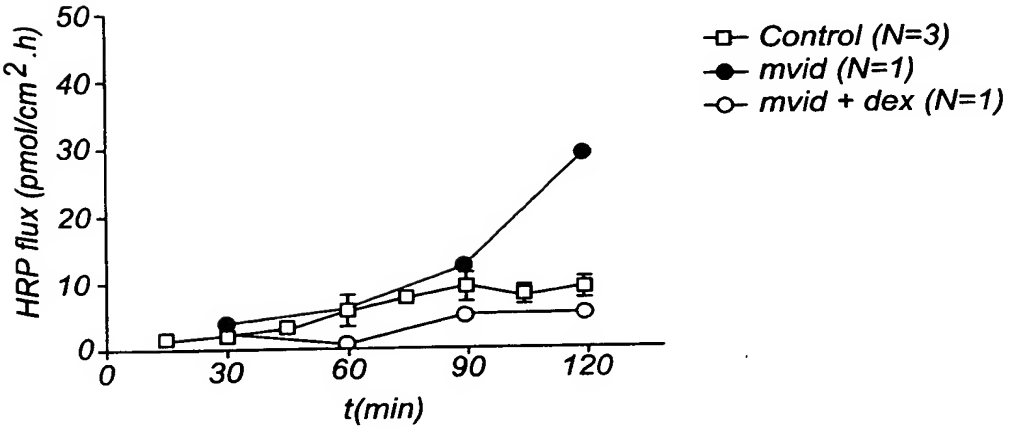

HRP
(pmol/cm 2)
180 min
20
10
0
C
D
C
D
1 hour ischaemia
1,5 hour ischaemia

Fig 6


HRP flux (pmol/cm 2 .h)
50
40
30
20
10
0
0
30
60
90
120
t(min)
Control (N=3)
mvid (N=1)
mvid + dex (N=1)

Voedingssamenstellingen die niet-verteerbare polysacchariden bevatten en gebruik ervan voor het verminderen van transport door tight junctions

De onderhavige uitvinding heeft betrekking op voedingssamenstellingen die een bepaalde klasse niet-verteerbare dextranen, gehydrolyseerde (galacto)mannanen en/of gehydrolyseerde (gluco)mannanen bevatten. Deze samenstellingen verminderen de opname door de darmwand van hoogmoleculaire stoffen, allergenen en micro-organismen. In het bijzonder betreft de onderhavige uitvinding het verminderen van het vrije transport van dergelijke stoffen door de tight junctions (TJ) van de darm, zonder dat het transport over het darmepitheel van laagmoleculaire stoffen zoals nutriënten, wordt belemmerd. De samenstellingen kunnen worden gebruikt om de, door verschillende oorzaken, verhoogde doorlaatbaarheid van de darmwand en de daardoor veroorzaakte penetratie van in het lumen aanwezige toxines, antigenen en pathogene micro-organismen te voorkomen.

De structuur en functie van tight junctions wordt onder andere beschreven in Ann. Rev. Physiol. 60, 121-160 (1998) en in Ballard T.S. et al., Annu.Rev.Nutr., 1995, 15:35-55. Tight junctions vormen geen starre barrière, maar spelen een belangrijke rol bij de diffusie door het darmepitheel van lumen naar bloedbaan en omgekeerd.

De doorlaatbaarheid van de tight junctions is sterk gereguleerd en kan worden verstoord door ziekte en bepaalde toxines in het lumen. De regulatie vindt plaats vanuit het zenuwstelsel, het hormonale systeem en het immuunsysteem. Bij het openen van de tight junctions zullen stoffen met een hoog molecuulgewicht, allergenen en zelfs micro-organismen de tight junctions passeren. De translocatie van stoffen met een hoog molecuulgewicht kan onder bepaalde voorwaarden sensibilisatie van het immuunsysteem opwekken en bij een volgende blootstelling allergische reacties tot gevolg hebben. Translocatie van pathogene micro-organismen legt een groot beslag op het immuunsysteem en kan, onder andere in perioden van verminderde weerstand, personen en dieren ziek maken. Hetzelfde geldt bijvoorbeeld voor bacteriële toxinen, die de epitheellaag hebben kunnen passeren en de bloedbaan hebben kunnen bereiken.

De uitvinding betreft nu het gebruik van een of meer niet-verteerbare polysacchariden gekozen uit de groep van dextranen met een molecuulgewicht van 8 kD tot 40.000 kD, gehydrolyseerde (gluco)mannanen met een molecuulgewicht van 0,5 kD tot 1000 kD en gehydrolyseerde (galacto)mannanen met een molecuulgewicht van

0,5 kD tot 1000 kD voor het verminderen van de opname door de darmwand van hoogmoleculaire stoffen, allergenen en micro-organismen, met dien verstande dat de door de polysacchariden veroorzaakte verhoging van de viscositeit van de voedingssamenstelling kleiner is dan 20 mPa.s.

Meer in het bijzonder betreft de uitvinding het gebruik van bovengenoemde samenstellingen voor het verminderen van transport van hoogmoleculaire stoffen, allergenen en micro-organismen door de tight junctions in de darm.

Naast het in belangrijke mate verminderen van transport van schadelijke stoffen en micro-organismen is een significant voordeel van de onderhavige uitvinding dat het normale transport van nuttige stoffen (nutriënten) zoals glucose, aminozuren, dipeptiden of sporenelementen nagenoeg gehandhaafd blijft.

Onder niet-verteerbare polysacchariden worden volgens de uitvinding polysacchariden verstaan die niet of nauwelijks verteerd of omgezet worden door de menselijke spijsverteringsenzymen onder de in het lichaam heersende omstandigheden. Opgemerkt dient te worden dat een deel van de niet-verteerbare polysacchariden wel gefermenteerd kan worden door de in de darm (colon, cecum en een deel van het ileum) aanwezige micro-organismen. Zonder aan enige theorie gebonden te willen zijn, wordt echter verwacht dat het effect van de polysacchariden op het paracellulaire transport niet via de fermentatieproducten plaatsvindt.

De mate waarin de polysacchariden verteerd worden, kan worden vastgesteld met de methode zoals beschreven in Minekus, M., Ph.D Thesis, Universiteit Utrecht, 1998, Development and validation of a dynamic model of the gastrointestinal tract, hoofdstuk 2. De polysacchariden volgens de uitvinding zijn minder dan 50% en bij voorkeur minder dan 30% verteerbaar.

Onder dextranen volgens de uitvinding worden via (bio)synthetische weg verkregen of natuurlijke dextranen verstaan. Het molecuulgewicht van dergelijke dextranen kan worden geregeld door gedeeltelijke zure of enzymatische hydrolyse van het molecuul gevolgd door herhaald fractioneren en precipiteren met alcohol of ultrafiltratie. Deze, op zich bij de vakman bekende, werkwijzen dienen zodanig te worden uitgevoerd dat het molecuulgewicht van de dextranen binnen het opgegeven traject van 8 kD tot 40.000 kD valt.

Bij voorkeur worden dextranen gebruikt met een molecuulgewicht van 20 kD tot 2000 kD.

Met (gluco)mannanen worden zowel de mannanen als de glucomannanen bedoeld. Dit geldt eveneens voor de (galacto)mannanen. Voorbeelden van galactomannanen zijn guargom, locust bean gum en taragum. Deze (galacto)- en (gluco)mannanen worden in gehydrolyseerde vorm toegepast. De molecuulgewichten liggen tussen 0,5 kD en 1000 kD.

Ook mengsels van dextranen, (galacto)mannanen en (gluco)mannanen kunnen worden toegepast.

De gehydrolyseerde (galacto) of (gluco)mannanen volgens de uitvinding kunnen verkregen worden door partiële, doch vergaande hydrolyse, bijvoorbeeld met behulp van daartoe geëigende enzymen waardoor substantiële hoeveelheden oligosacchariden ontstaan met een ketenlengte van 3 tot 5600, bij voorkeur van 4 tot 1000.

De polysacchariden zijn bij voorkeur in een dusdanige hoeveelheid in het preparaat aanwezig dat de concentratie van deze polysacchariden in de darm 0,1 tot 20 g/l, bij voorkeur 0,5 tot 10 g/l, het liefst 1 tot 6 g/l is. De minimale hoeveelheid van de werkzame stof wordt bepaald doordat een significante afname van het transport door de tight junctions wordt waargenomen.

Het is niet noodzakelijk dat de polysacchariden worden toegediend op die plek waar het paracellulaire transport verstoord is. Het aanwezig zijn van de actieve component op een plek ergens in de darm tussen de maag en de aangedane plek is voldoende.

Sommige van de volgens de uitvinding gebruikte polysacchariden hebben een viscositeitverhogende werking, die de absorptie van voedingscomponenten zou kunnen verhinderen. Het preparaat moet een zodanige samenstelling hebben dat het normale transcellulaire transport niet belemmerd wordt.

Meer in het bijzonder heeft de voedingssamenstelling volgens de uitvinding een viscositeit van kleiner dan 100 mPa.s, bij voorkeur kleiner dan 40, met nog meer voorkeur kleiner dan 30 mPa.s. Het is bij de onderhavige uitvinding met name van belang dat de polysacchariden, onafhankelijk van de overige bestanddelen van de samenstelling, slechts een gering viscositeitverhogend effect hebben. Het viscositeitverhogende effect van de werkzame polysacchariden in de samenstelling moet minder dan 20 en liever minder dan 10 mPa.s bedragen en kan bijvoorbeeld 3 mPa.s zijn. De viscositeit van het product wordt dus voor het grootste deel veroorzaakt door andere componenten in het product dan de polysacchariden.

De viscositeit wordt bepaald door middel van een Carri-med bij een afschuifsnelheid van 100 per seconde en bij 20 °C.

Bij droge producten gelden de hiervoor beschreven grenzen voor de viscositeit na reconstitutie van het product.

In het algemeen zullen derhalve het type polysaccharide (molecuulgewicht) evenals de concentratie daarvan zodanig worden gekozen dat een optimale combinatie van werkzaamheid en viscositeit verkregen wordt. Niet alleen molecuulgrootte, maar ook vertakkingsgraad en beladingsgraad bepalen werking, viscositeit en/of fermentatiegedrag.

De polysacchariden volgens de uitvinding verhinderen het vrije transport van hoogmoleculaire stoffen, allergenen en micro-organismen door de tight junctions van de darmwand. Onder hoogmoleculaire stoffen wordt in dit verband verstaan de stoffen die onder normale omstandigheden de tight junctions niet, of slechts in geringe hoeveelheden, kunnen passeren en waarvan een toxische of allergene werking kan worden verondersteld. Deze zullen over het algemeen een molecuulgrootte boven 4000 Dalton hebben. Antigenen, stoffen die het immuunsysteem activeren, zijn in het algemeen peptiden, al dan niet geglycosideerd, vaak met een molecuulgewicht boven de 10.000 Dalton. Allergenen zijn antigenen die een allergische reactie teweeg brengen, die veelal via immuun-globuline E gemedieerd wordt.

Onder micro-organismen worden in dit verband met name micro-organismen verstaan die in het darmlumen voorkomen. Zo kan bijvoorbeeld onder bepaalde omstandigheden overgroei plaatsvinden van micro-organismen in de dunne darm, waardoor tight junctions in verhoogde mate worden blootgesteld aan deze micro-organismen.

Volgens een ander aspect van de uitvinding worden voedingen of preparaten voorgesteld die deze niet-verteerbare polysacchariden bevatten. Deze voedingen kunnen zijn:

- complete voedingen;
- voedingssupplementen;
- gezondheidbevorderende preparaten; en
- sondevoedingen.

De samenstellingen volgens de uitvinding kunnen worden gebruikt ter voorkoming of ter behandeling van bepaalde vormen van allergie, allergische reacties,

sepsis en inflammatoire processen, zoals die op kunnen treden bij emotionele en fysieke stress, ischaemie, reperfusieschade tijdens en na operaties, na bestraling en/of chemotherapie van kankerpatiënten en bij inflammatoire darmziekten diarree en allergieën.

De hiervoor beschreven complete voedingen en voedingssupplementen kunnen in het bijzonder worden gebruikt bij de behandeling of ter voorkoming van inflammatoire darmziekten, zoals colitis ulcerosa, Inflammatory Bowel Disease en de ziekte van Crohn. Specifieke andere bestanddelen die in dergelijke voedingen en supplementen kunnen worden opgenomen, zijn groeihormonen, glutamine, n-3 LCPUFA's en de vereiste gehaltes aan macro- en micro-ingrediënten.

Voorts kunnen de voedingen volgens de uitvinding worden toegepast voor en na operaties. Bij operaties treedt namelijk vaak ischaemie en reperfusieschade op aan de darm waardoor de tight junctions zich openen. Het voor en na de operatie in de darm brengen van de polysacchariden volgens de uitvinding zou het ongecontroleerde paracellulaire transport kunnen voorkomen. Ook na chemotherapie kan het toedienen van deze polysacchariden gunstig zijn.

Bij diarree kunnen ook een aantal patho-morphologische veranderingen optreden die gepaard gaan met een verhoogde permeabiliteit van de tight junctions. Deze veranderingen kunnen optreden bij bepaalde vormen van diarree. De complete voedingen en voedingssuplementen volgens de uitvinding kunnen voor het tegengaan van de nadelige gevolgen van deze verhoogde permeabiliteit gebruikt worden.

Ook tijdens stress, zowel van fysieke aard (bijvoorbeeld duursporten) als van emotionele aard, kunnen de tight junctions zich openen waardoor bacteriële translocatie plaatsvindt. Voorbeelden van emotionele stress waarbij dit plaatsvindt is de stress die optreedt tijdens het transport van varkens naar het slachthuis. Hierdoor kan besmetting van het vlees optreden. Een ander voorbeeld is de stress die optreedt bij het afspenen (weaning) van biggen. Voor, tijdens of na het plaatsvinden van de stress kunnen de polysacchariden toegediend worden.

Met behulp van de polysacchariden volgens de uitvinding kunnen eveneens preparaten worden bereid, die geschikt zijn voor patiënten met een voedselallergie, zoals een allergie voor koemelk of voor gluten. De toename van de doorlaatbaarheid door blootstelling aan het allergeen kan voorkomen worden. Deze preparaten worden zodanig samengesteld dat hierin niet de genoemde allergenen aanwezig zijn.

De uitvinding wordt toegelicht aan de hand van de nu volgende voorbeelden en aan de hand van de bijgevoegde figuren, waarin

Figuur 1 een weergave is van de in de voorbeelden toegepaste Ussingkamer;

Figuur 2 de remming van het effect van capraat door dextraan weergeeft;

Figuur 3 de remming door gehydrolyseerde taragum van de door mellitin verhoogde para-cellulaire doorlaatbaarheid weergeeft;

Figuur 4 het effect van dextranen op de HRP flux weergeeft;

Figuur 5 het effect van dextraan op de HRP flux bij een varken onder verdoving weergeeft;

Figuur 6 de invloed van dextraan op de verhoogde permeabiliteit van de darm van een microvillus inclusion patient weergeeft.

Voorbeelden

I. Voorbeelden van producten

Hierna worden voorbeelden gegeven van samenstellingen van verschillende typen producten waarin de actieve component dextraan is.

De verschillende typen product kunnen complete enterale voedingen, voor gebruik door de patiënt zelf of voor gebruik als sondevoeding zijn. Het product kan zowel een vloeibare vorm als poedervorm hebben, die na oplossen klaar voor gebruik is. De actieve componenten kunnen ook als ingrediënt in een ander voedingsmiddel gebruikt worden (bijvoorbeeld brood) of in voedingssupplementen, zoals een reep, een zuivelproduct, zoals yoghurt, of een poeder in de vorm van een sachet.

Voorbeeld 1

Ready to feed, vloeibare, complete voeding voor gebruik voor of na operaties. Per 100 ml van het product bevat de samenstelling:

Eiwit:	7,0 g
Vet:	4,0 g
Koolhydraten:	21 g
Dextraan :	0,2 g

Per 100 ml van het product worden mineralen toegevoegd in een hoeveelheid van 1/15 deel van de aanbevolen dagelijkse hoeveelheid (=ADH). Sporenelementen en

vitamines worden toegevoegd in iets hogere hoeveelheden, te weten 2/15 ADH. Het product is zodanig samengesteld dat 1500 ml geconsumeerd dient te worden door de patiënt.

Voorbeeld 2

Complete sondevoeding voor personen die lijden aan Inflammatory Bowel Disease. Per 100 ml bevat het product:

Eiwit op basis van caseïne 7,0 g

Vet op basis van plantaardige oliën en 10% visolie en 20% MCT; het gehalte linolzuur = 20 % en dat van alfa-linoleenzuur = 4,5%

Premixen met de gebruikelijke vormen van sporenelementen, vitamines en mineralen Na, K, Ca, Mg, P, Zn, Fe, Mn, Cu, Vit. B1, B2, niacine, A, D, K, B6, B12, pantotheenzuur, foliumzuur

Dextraan: 0.6 g

Voorbeeld 3

Voedingssupplement voor patiënten die lijden aan voedselintolerantie of allergie

Yoghurt op basis van sojamelk. Per 100 ml bevat de yoghurt:

Eiwit 4,0 g, vet 3,9 g, koolhydraten 12,3 g en 0,1 ADH aan vitamines en sporenelementen

Na =80; K=135; Cl=125; Ca=50; P=50; Mg=20 mg

Gehydrolyseerde galactomannanen 0,5 g

Voorbeeld 4

Energiedrank voor sporters

Per 100 ml bevat de vloeistof

Koolhydraten:	7,0 g
Glucose:	0,2 g
Fructose:	1,8 g
Lactose:	0,4 g
Saccharose:	1,7 g
Polysacchariden:	2,5 g

Organische zuren:	0,4 g
Mineralen:	
Na:	37 mg
K:	17 mg
Cl:	58 mg
Ca:	8 mg
Mg:	1 mg
Vitamine C:	15 mg
Dextraan:	0.1 g

Voorbeeld 5

Premix voor gebruik in varkens- of biggenvoer

A/Premix bestaande uit 90% maïsmeel en 10% dextraan 150 kD

B/Premix bestaande uit een geschikte premix van vitamines, sporenelementen en mineralen en 10 % dextraan.

Premix A, B of mengsels hiervan kunnen worden gebruikt bij de vervaardiging van varkensvoeders. Dit kunnen speciaalvoeders zijn voor gebruik als varkens op transport worden gezet, herschikt moeten worden in de stal of indien ze een periode van verminderde weerstand hebben.

Ook kunnen de premixen gebruikt worden in een biggenvoer voor gebruik na spenen, als additief of in plaats van de premixen die reeds bekend zijn voor gebruik in biggenvoer.

II. Effect op transport via de tight junctions van de darm

Voor het bepalen van de werking van de gebruikte polysacchariden werd gebruik gemaakt van een modelopstelling.

Een proefdier, zoals een rat of cavia, wordt onder narcose gebracht. Vervolgens wordt de buikwand geopend en een stuk van het ileum wordt afgebonden. Het darmweefsel wordt uitgenomen en ontdaan van spierlagen. Het zo verkregen preparaat wordt daarna gespannen tussen twee met geoxygeneerde oplossingen doorstroomde compartimenten (Figuur 1). Het preparaat werd behandeld met hetzij buffer (controle of nulwaarde) of capraat in buffer om de tight junctions open te zetten (100 % permeabiliteit), hetzij de combinatie van capraat en een bepaalde concentratie

polysaccharide in buffer. Als maat voor de permeabiliteit wordt het transport van HRP (horse radish peroxidase) over het preparaat gemeten volgens bekende methoden.

De resultaten van dit type experimenten worden weergegeven in figuur 2 tot en met 5.

In Figuur 2 is het in vitro effect van dextraan (70 kD) op de door capraat verhoogde HRP flux in cavia darmepitheel weergegeven.

In Figuur 3 is het in vitro effect van taragumhydrolysaat (900D) op de HRP flux van Caco-2-cellen weergegeven o.i.v. 2 μM mellitin. Er blijkt dat de door mellitin verhoogde paracellulaire doorlaatbaarheid wordt geinhibeerd door taragum.

In Figuur 4 is het effect weergegeven van verschillende dextranen op de HRP flux van Caco-2-cellen o.i.v. 2 μM mellitin. In de figuur staat Phar. voor Pharmacosmos.

In Figuur 5 wordt het effect van dextraan op de door ischemie verhoogde HRP flux in varkensdarm weergegeven. Het betreft experimenten met een varken onder volledige narcose, waarbij segmenten van het caudale deel van het jejenum werden genomen. Het effect van 5,6 g/l dextraan (70 kD) (D) in het in situ ischemie-reperfusie model in varkens in afhankelijkheid van duur van ischemie in vergelijk met controle (C) waarbij niet toegevoegd is in het lumen tijdens ischemie. Er wordt een significante daling van de HRP flux onder invloed van dextraan gevonden ten opzichte van de controlewaarde.

Van een kind dat lijdt aan Microvillus Inclusion Disease (MVID) werden zuig-biopten van het duodenum genomen. Deze preparaten vertoonden in de Ussingkamer een viervoudig verhoogde doorlaatbaarheid voor HRP ten opzichte van de normaalwaarde. Na toevoeging van dextranen van 70 kD tot een concentratie van 4,2 g/l aan het luminale compartiment in de Ussingkamer werd de doorlaatbaarheid gereduceerd tot het normale niveau. Met electronenmicroscopie kon geen HRP meer worden aangetroffen in de paracellulaire ruimten of tight junctions. Een overeenkomstig resultaat werd verkregen met dextranen met een molecuulgewicht van 150 kD.

Figuur 6 toont het resultaat van dit experiment met dextranen met een molecuulgewicht van 70 kD. Na 120 minuten is een duidelijk verschil waarneembaar in de doorlaatbaarheid met en zonder toevoeging van dextranen.

Conclusies

1. Gebruik van een of meer niet-verteerbare polysacchariden gekozen uit de groep van dextranen met een molecuulgewicht van 8 kD tot 40.000 kD, gehydrolyseerde (gluco)mannanen met een molecuulgewicht van 0,5 kD tot 1000 kD en gehydrolyseerde (galacto)mannanen met een molecuulgewicht van 0,5 kD tot 1000 kD voor de bereiding van een voedingssamenstelling voor het verminderen van de opname door de darmwand van hoogmoleculaire stoffen, allergenen en micro-organismen, met dien verstande dat de door de polysacchariden veroorzaakte verhoging van de viscositeit van de voedingssamenstelling kleiner is dan 20 mPa.s.

2. Gebruik volgens conclusie 1, waarbij de polysacchariden worden gekozen uit dextranen met een molecuulgewicht van 20 kD tot 2000 kD.

3. Gebruik volgens een van de voorgaande conclusies, waarbij de polysacchariden in de samenstelling aanwezig zijn in een dusdanige hoeveelheid dat de concentratie van deze polysacchariden in de darm 0,1 tot 20 g/l, bij voorkeur 0,5 tot 10 g/l, het liefst 1 tot 6 g/l is.

4. Gebruik volgens een van de voorgaande conclusies, waarbij de voedingssamenstelling de vorm heeft van een complete voeding.

5. Gebruik volgens een van de conclusies 1 tot 3, waarbij de voedingssamenstelling de vorm heeft van een voedingssupplement.

6. Gebruik volgens een van de voorgaande conclusies, voor het verminderen van transport van hoogmoleculaire stoffen, allergenen en micro-organismen door de tight junctions in de darm.

7. Gebruik volgens een van de voorgaande conclusies, ter voorkoming of ter behandeling van allergie, allergische reacties, sepsis en inflammatoire processen, zoals die op kunnen treden bij emotionele en fysieke stress, ischaemie, reperfusieschade tijdens en na operaties, na bestraling en/of chemotherapie van kankerpatiënten en bij inflammatoire darmziekten, diarree en allergieën

8. Voedingssamenstelling die dextranen met een molecuulgewicht van 8 kD tot 40.000 kD bevat, met dien verstande dat de door de dextranen veroorzaakte verhoging van de viscositeit van de voedingssamenstelling kleiner is dan 20 mPa.s.

Uittreksel

De onderhavige uitvinding heeft betrekking op het gebruik van een of meer niet-verteerbare polysacchariden gekozen uit de groep van dextranen met een molecuulgewicht van 8 kD tot 40.000 kD, gehydrolyseerde (gluco)mannanen met een molecuulgewicht van 0,5 kD tot 1000 kD en gehydrolyseerde (galacto)mannanen met een molecuulgewicht van 0,5 kD tot 1000 kD voor de bereiding van een voedingssamenstelling voor het verminderen van de opname door de darmwand van hoogmoleculaire stoffen, allergenen en micro-organismen, meer het bijzonder voor het verminderen van transport van hoogmoleculaire stoffen, allergenen en micro-organismen door de tight junctions in de darm., waarbij de door de polysacchariden veroorzaakte verhoging van de viscositeit van de samenstelling kleiner is dan 20 mPa.s.

De voedingssamenstellingen kunnen worden toegepast ter voorkoming of ter behandeling van allergie, allergische reacties, sepsis en inflammatoire processen, zoals die op kunnen treden bij emotionele en fysieke stress, ischaemie, reperfusieschade tijdens en na operaties, na bestraling en/of chemotherapie van kankerpatiënten en bij inflammatoire darmziekten, diarree en allergieën.